



# Genetic management of mixed-stock fisheries “real-time”: The case of the largest remaining cod fishery operating in the Atlantic in 2007–2017



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## ABSTRACT

Fish stocks represent fundamental units in fisheries management, and their identification, especially in mixed-fisheries, represents one of the primary challenges to sustainable harvest. Here, we describe the first “real-time” genetic management program used to manage a mixed-stock fishery of a non-salmonid and commercially significant marine fish, the Atlantic cod (*Gadus morhua* L). Based upon the analysis of > 18 000 fish sampled from the commercial catch in Lofoten (Norway), which represents the largest remaining cod fishery in the Atlantic, we estimated the fraction of North East Arctic cod (NEAC), and Norwegian Coastal cod (NCC), just 24 h post-landing. These estimates, based upon the analysis of the *Pantophysin* gene, were performed weekly in the winter and spring of each year in the period 2007–2017. The program has successfully permitted the Norwegian Directorate of Fisheries to actively manage the commercial exploitation of the highly abundant NEAC stock, while simultaneously limiting exploitation of the fragile NCC stock, both of which overlap at the spawning grounds. Data from this program have also revealed a distinct temporal increase in the fraction of NEAC on the spawning grounds in this region, which is consistent with the overall increased abundance of this stock as estimated by ICES.

## 1. Introduction

Harvest from the world’s oceans has remained stable between 80–90 million tonnes/annum since the mid-1980s, and many of the world’s fisheries are either fully or over-exploited, depleted, or recovering from depletion (FAO, 2016). In addition, illegal, unreported and unregulated (IUU) fishing represents a major challenge to the sustainable harvest of marine resources (Agnew et al., 2009). Increasing the sustainability of harvest from the marine realm is vitally important given the current state of many fisheries, and the continued increase in the human population and its expanding requirements for food.

DNA methods provide unprecedented knowledge of population genetic structure for many of the exploited marine resources, including fish. In many cases, independent stocks and management units within fisheries have been identified using this approach (Hauser and Carvalho, 2008; Reiss et al., 2009). There are also examples of genetic methods being implemented in the active regulation of fisheries (Nielsen et al., 2001; Ogden, 2008; Glover, 2010; Glover et al., 2012;

Flannery et al., 2010). Nevertheless, widespread integration of genetic data into fishery policy has been slow, and explicit and quantitative inclusion of genetic data into fisheries models is still relatively rare (Waples et al., 2008; Reiss et al., 2009).

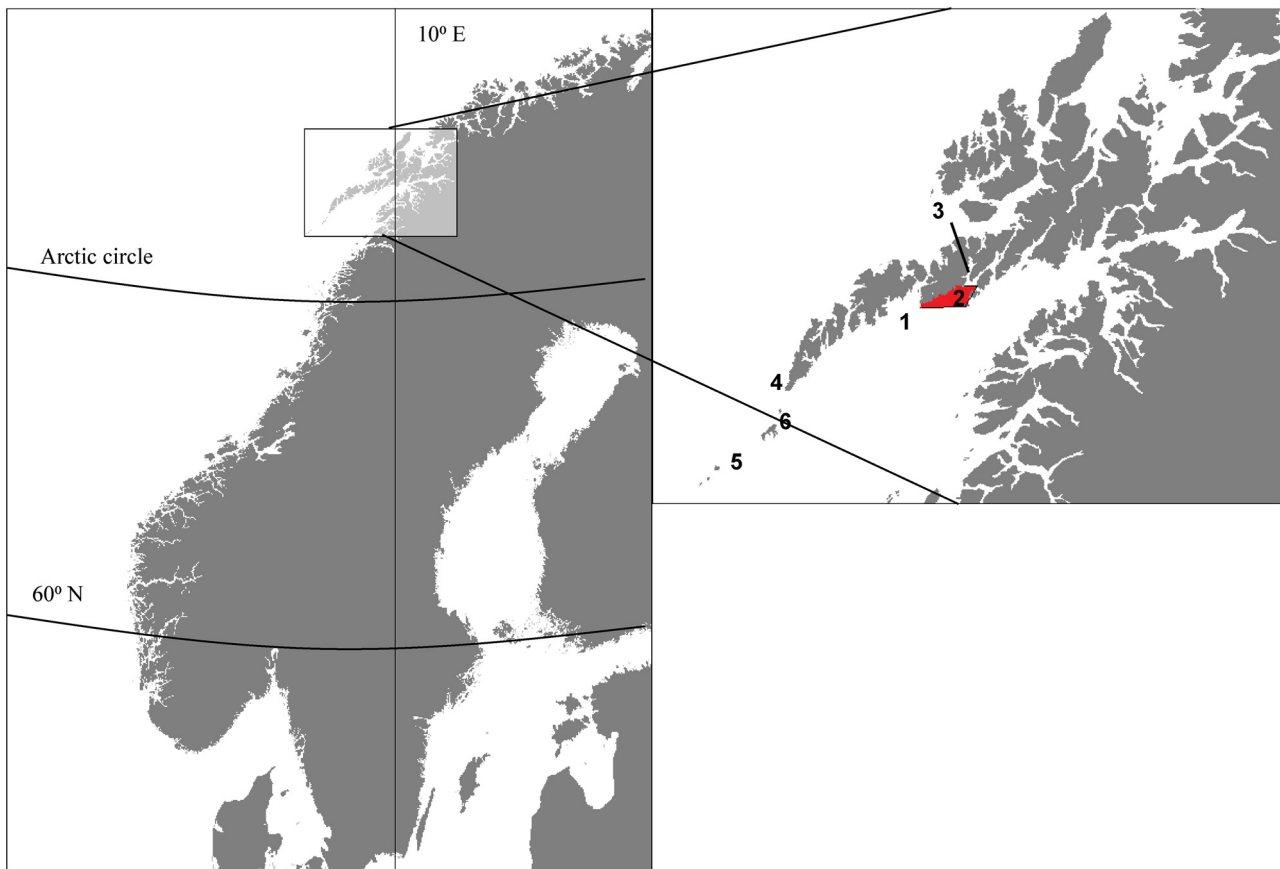
One of the challenges to sustainable fisheries management is when two or more stocks, that are morphologically very similar or identical and thus impossible to differentiate in the fishery, overlap in time and space. In such cases, harvesting potentially leads to under- and over-exploitation of the separate components of the fishery (Allendorf et al., 2008). Mixed stock fisheries may occur where separate populations partially or completely overlap in their spawning areas. This is for example the case for the Northeast Atlantic cod (*Gadus morhua*) (NEAC) and Norwegian coastal cod (NCC) which have and continue to form the basis of major fisheries along the coast of Norway, and especially the Lofoten Islands (Fig. 1). Mixed stock-fisheries may also occur when multiple populations overlap on the feeding grounds. For example, Atlantic salmon (*Salmo salar* L.) originating from multiple distinct populations on both the west and east Atlantic meet on the high seas and

Abbreviations: IUU, illegal, unreported and unregulated; ICES, International Council for Exploration of the Sea; NEAC, North East Arctic cod; NCC, Norwegian coastal cod; IMR, Institute of Marine Research; NDF, Norwegian Directorate of Fisheries

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**Fig. 1.** Sampling areas in Lofoten. 1 – Henningsværstraumen, 2 – Henningsværboksen, 3 – Austnesfjorden, 4 – Bleiksegga, 5 – Røst, 6 – Værøy.

have been historically exploited in the fishery operated around the Faroe Islands (Gilbey et al., 2017). A similar situation exists for the many salmonid fisheries in the Northeast Pacific. Here, in contrast to the Atlantic salmon fishery around the Faroe Islands which has been suspended (ICES, 2016), oceanic salmon fishing is permitted despite capturing fish from multiple genetically distinct stocks. However, the fisheries are actively regulated with genetic methods to ensure that a sufficient number of adult salmon return to each river to ensure the river's spawning target is achieved (Seeb et al., 2004; Flannery et al., 2010). Similar approaches could be used to monitor other marine fisheries where possible.

Historically, the Atlantic cod has formed the basis of many economically significant fisheries operating on both sides of the Atlantic. However, over-exploitation in many regions has left highly depleted stocks and multiple fishery collapses. The best documented example of this being the total collapse of the cod stock in the Grand Banks fishery located off east Canada (Hutchings and Myers, 1994). In Norway, the numbers of NCC have also severely declined (ICES, 2003, 2014), however, the NEAC stock has remained relatively stable. NEAC undertakes long-distance migrations to the feeding grounds in the Barents Sea but spawn in coastal regions of Norway, primarily in the Lofoten and Møre area (Fig. 1, Bergstad et al., 1987; Sundby and Nakken, 2008). In contrast to NEAC, NCC displays a limited migratory behaviour, remaining in coastal areas throughout its life (Jakobsen, 1987; Svåsand, 1990; Michalsen et al., 2014). However, just like NEAC, NCC spawns in coastal regions of Norway, including the Lofoten area (Hysten, 1964; Berg and Albert, 2003). Therefore, these different stock components with different abundances may be observed on the same spawning grounds at the same time (e.g., Johansen et al., 2017). In turn, this creates a significant challenge for the sustainable exploitation of NEAC, while protecting NCC in that area.

NCC has been recognised as different from NEAC for more than 80

years (Rollefsen, 1933), and since 2001, ICES has provided management advice for coastal cod in the area north of 62°N. In the annual quota agreements between Norway and Russia since 2005, however, an expected catch of NCC has been added annually to the Norwegian NEAC quota. From the mid-1970s, until 2003, an expected annual catch of 40 000 t NCC was added to the 5–10 times bigger quota for NEAC. The total quota was thereafter driven primarily by the status of the NEAC stock, leading to an inherent risk of over-exploiting NCC. Due to the decline of NCC, ICES advised a zero catch of NCC for the years 2004–2011, and at the same time recommended establishing a recovery plan (ICES, 2003, 2014). However, stopping all commercial exploitation of NCC would require a closure of all coastal fisheries in Norway where NCC also formed part of the catch. As this was not considered feasible, the expected catch of NCC (still included in the total Norwegian cod quota) was reduced from 40 000 to 20 000 t, and technical regulations aimed at reducing NCC catches (and by-catches) were introduced. Instead of enforcing a separate quota for NCC the Norwegian authorities chose to reduce the fishing pressure on NCC by means of technical regulations. These included moving fishing effort from areas and seasons where NCC dominated the catches, to areas and seasons where NEAC dominated. One of the regulatory measures included closing all commercial fishery activities in one important NCC spawning area in Lofoten (“Henningsværboksen”) during the spawning season (Fig. 1). However, this was done on the premise that if the sampling of catches along the border of the area proved that the fraction of NEAC was high, the authorities would consider a temporary reopening for fishery, under the argument that it is better to allow the fleet to fill their quota by NEAC rather than catching in NCC during other times of the year. Therefore, a sampling program was needed.

In Norway, otoliths have and continue to be used to differentiate between NEAC and NCC (Rollefsen, 1933). However, accurate otolith typing is dependent on trained personnel (Berg et al., 2005).

Furthermore, fishing with one of the most common gears in Norwegian fisheries, i.e. gill-nets, involves heading and gutting the catch prior to landing. This limits the ability to use otolith typing as a rapid method to routinely monitor the NEAC and NCC components of the fishery. The discovery of the Pantophysin locus (*PanI* previously called *SypI*) (Fevolden and Pogson, 1997), which exhibits close to diagnostic allele frequency differences between NEAC (*PanI*<sup>B</sup> allele 0.90) and NCC (*PanI*<sup>A</sup> allele 0.81) (Fevolden and Pogson, 1997; Sarvas and Fevolden, 2005), provides an alternative to otolith typing. In addition, there is a strong correlation between the genotype at this locus and otolith category (Wennevik et al., 2008). Furthermore, given that this single gene is rapidly genotyped on relatively simple equipment, it provides the ability to analyse a catch and compute the NEAC/NCC fractions rapidly – also on a catch where the head and thus otoliths have already been removed before landing.

In 2005, the Institute of Marine Research (IMR) conducted a pilot study in Lofoten to evaluate the logistical feasibility of genotyping the *PanI* locus to estimate the fractions of NEAC and NCC from commercial landings in this economically important fishery “real-time” – i.e., within 24 h post-sampling. Based upon this pilot study, a genetics-based fishery program was established in cooperation with the Norwegian Directorate of Fisheries (NDF) to control the annual fishery in this area in the late winter and early spring. The overall aim of the program was to actively manage the fishery in the inner part of the Lofoten Islands, thus permitting commercial harvest of NEAC while simultaneously restricting harvest of NCC. Based upon this overall aim, the NDF set an arbitrary threshold of a minimum of 70% NEAC among the catch in this region in order to open, and hold open the fishery in the closed area, “Henningsværboksen”, to commercial vessels using gill-nets. Here, we present the results of this fisheries regulation program that has run annually in the period 2007–2017 and includes the weekly-estimation of NEAC fraction in the commercial catch in 200 independent samples based upon the analysis of the *PanI* locus in > 18 000 cod.

## 2. Materials and methods

### 2.1. Sampling the fishery

All of the samples upon which the present study is based were collected from fish captured by commercial fishing vessels in the Lofoten fishery. All samples were taken from dead fish by officers of the NDF who are responsible for the regulation of the fishery, and routinely perform inspections at the different landing ports. Consequently, no licences were required to obtain the genetic samples upon which the study is based.

Each year, samples of the catch were taken weekly in the period February until the end of the fishing season in May. The exact period of sampling varied among years, and in the period 2015–2017, samples were only taken at the start of the season due to the dramatically increased fraction of NEAC during the 11-year study period (see results). Each week, fin clips from 96 fish were collected from the unsorted catch on arrival at port, placed in 96-well plates, and sent to the IMR for immediate genetic analysis. Commercial vessels partaking in the Lofoten fishery use a range of commercial gear, predominantly Danish seine and gill-nets. However, most of the samples were taken from vessels that had used bottom gill nets (Supplementary file A1). This is because catches from vessels using gill-nets are typically landed earlier in the day, thus permitting the samples to be taken and posted to the analytical laboratory on the same day. Results of the genetic analysis were delivered back to the NDF within 24 h of the samples being taken.

Based upon the sampling regime described above, a total of 18 291 cod, representing ~200 independent samples, were collected from the Lofoten fishery through the 11-year study period (2007–2017) (Table 1, Supplementary file A1). Individuals were primarily sampled from two areas; Henningsværstraumen and Austnesfjorden until 2012 (Fig. 1), when we in addition started collecting samples in the restricted-fishing

**Table 1**

Summary of number of samples, individuals and DNA analysis success rate.

Year	Lofoten			
	# samples (Henningsværboksen)	# individuals	DNA isolated <sup>a</sup>	%DNA <sup>b</sup>
2007	18 (1)	1597	1529	95.7%
2008	25 (2)	1849	1622	87.7%
2009	17	1632	1624	99.5%
2010	20	1888	1822	96.5%
2011	26	2496	2233	89.5%
2012	34 (11)	3264	2847	87.2%
2013	29 (13)	2784	2517	90.4%
2014	10 (5)	776	707	91.1%
2015	7 (4)	661	639	96.7%
2016	7 (3)	672	650	96.7%
2017	7 (4)	672	658	97.9%
<b>Total</b>	<b>200</b>	<b>18 291</b>	<b>16 848</b>	<b>92.1%</b>

<sup>a</sup> Number of individuals that produced readable results.

<sup>b</sup> %DNA is the fraction of samples that produced readable results.

**Table 2**

Samples collected outside the main spawning area in Lofoten.

Location	Date	N	Fraction NEAC
Bleiksegga	3/7/2007	89	99.4%
Røst	3/7/2007	91	100%
Værøy	3/7/2007	91	94.2%
Røst	3/22/2008	70	90.8%

zone in Lofoten (“Henningsværboksen”) (See Supplementary file A1).

In order to quantify the frequency of NEAC on the outer coastal area of this region, a small number of additional samples (351 fish) were also analysed from 2007 and 2008 (Points 4–6 in Fig. 1, Table 2). These are treated independently of the main data set and did not form part of the management regime. These samples were also taken from dead fish captured as part of the commercial catch.

### 2.2. Genetic analysis

Throughout the 11-year study period, DNA isolation and analysis of the *PanI* locus was performed in 96-well format using slightly different methods. In the first two years (2007–2008), DNA was isolated using Chelex beads (Bio-Rad Laboratories). In brief, a small piece of tissue was put in 100  $\mu$ l 5% Chelex solution (5% Chelex, 10 mM Tris, 1 mM EDTA and 1% SDS) and 3  $\mu$ l of Proteinase K (25  $\mu$ g/ml) was added. The isolation mixture was placed at 55 °C over-night and followed by a 15-min incubation at 95 °C. DNA was diluted 40-fold for PCR amplification using the *PanI* primers (Nielsen et al., 2007). PCR products were thereafter digested with the restriction enzyme EcoRI, and alleles identified using agarose gel electrophoresis.

In the second period (2009–2017), DNA isolation was performed using the HotSHOT method (Truett et al., 2000). In brief, this method is based upon heating (95 °C) the samples in Sodium hydroxide (25 mM) for 30 min, neutralizing the sample with Tris buffer, and then using 2  $\mu$ l of the solution directly for the PCR reaction. In this period, primers constructed for an ABI sequencer and Real-Time PCR (Stenvik et al., 2006), producing two different fragments, either two 85 bp fragments labelled with 6-FAM fluorophore–*PanI*<sup>A</sup>, two 87 bp fragments labelled with PET fluorophore–*PanI*<sup>B</sup>, or the heterozygote producing both fragments, were used for analysis of *PanI*. The different fragments were subsequently separated using an ABI 3100 XL sequencer (Applied Biosystems) and scored with the GeneMapper software (Applied Biosystems).

### 2.3. Statistical analysis

The fraction of NEAC in each sample (*Fraction NEAC*) was estimated by the equation:

$$\text{Fraction NEAC} = (\text{Fraction B} - \alpha) / (1 - 2\alpha)$$

*Fraction B* is the observed fraction of the *PanI* allele B in the sample, and  $\alpha$  is the assumed *Fraction B* in a “pure” coastal cod sample. Previous results from a pilot experiment in the Lofoten area (Wennevik et al., 2008) indicated  $\alpha$  close to 0.10, while other samples from fjords along the Norwegian coast indicated a lower  $\alpha$ , close to 0.05. To quantify the associated uncertainty range, the *Fraction NEAC* for each sample was calculated for both values of  $\alpha$ , while the average of the two estimates was reported as the “best estimate”.

## 3. Results

### 3.1. Analytical success

The average success rate of the genetic analysis, measured as produced PCR fragments, averaged between 87.2 and 99.5% per year across the 11-year period. This included handling > 18,000 cod within 24 h of being landed by a commercial vessel. The number of individuals and success rate of DNA isolation for all years are presented in Table 1, and at the specific sample level in Supplementary file A1.

### 3.2. Weekly trends

Based upon the samples successfully analysed as described above, the weekly fraction of NEAC in the catch was estimated (Fig. 2, Supplementary file A1). Depending on the year and exact location sampled, in general, the fraction of NEAC in the commercial catch increased during the period February–March, peaked in the period late March early April, and decreased thereafter (Fig. 2). The rate of increase in the estimated fraction of NEAC in the weekly catch during the fishing season varied among years. For example, the first sample taken on 16th February 2009 contained no NEAC in the catch in Henningsværstraumen, but this increased sharply in the following weeks to a high of ~50–60% in the last and first weeks of March and April respectively. In contrast, the first sample taken in the last week of February in Henningsværstraumen in 2013 already contained an estimated fraction of nearly 80% NEAC.

Differences in the estimated fraction of NEAC also varied among sampling localities. For example, in some of the early years of the management program, where the estimated fraction of NEAC in the catch was relatively low or modest, the NEAC fraction was greater in Henningsværstraumen than in the inner area Austnesfjorden (see for example years 2009 and 2010 for illustration – Fig. 2). In contrast, in years with a higher overall fraction of NEAC estimated in the catch, differences between Henningsværstraumen and the inner area, Austnesfjorden, were less clear (see for example years 2011, 12 and 13 Fig. 2).

The observed differences in the estimated fraction of NEAC between Henningsværstraumen and the inner area, Austnesfjorden, are to a certain degree also mirrored in the data from the reference samples collected in the outer-island areas of Lofoten at Røst, Værøy and Bleiksegga (Fig. 1, sites A4–A6). At these locations sampled on one single occasion in March 2007 and 2008, the fraction of NEAC was estimated to be between 90.8 and 100% (Table 2). In the same month, the fraction of NEAC estimated in Henningsværstraumen was only ~50% and ~20% in 2007 and 2008 respectively (although this jumped a bit on a weekly basis), and < 10% and ~30% in 2007 and 2008 respectively for the inner sampling location Austnesfjorden. Collectively, these data indicate that there is a higher fraction of NEAC in the outer areas, which tends to flux into the inner areas with a small time-delay.

In addition, the absolute fraction of NEAC arriving in the inner areas appears to be dependent upon the number of NEAC arriving to the area. I.e., a greater fraction of NEAC was reported in the innermost sampling area Austnesfjorden in years when the total catch was highest.

### 3.3. Yearly trends and alteration in the management regime

As indicated above, large yearly variations in the estimated fraction of NEAC in the sampling areas Henningsværstraumen and Austnesfjorden were observed. When these data were aggregated per year, a clear trend showing an increase in the overall fraction of NEAC in the landings inside the Lofoten Islands was observed (Fig. 3a). This overall increase in NEAC fraction with time is to some degree mirrored in the ICES estimate of NEAC abundance (Fig. 3a), and to a greater degree the increased total harvest in this area (Fig. 3b). Thus, the increased fraction of NEAC observed in this area during the study period, to a certain degree reflects the increased abundance of NEAC in general.

Based upon the increase in the estimated fraction of NEAC in the catches in Henningsværstraumen and Austnesfjorden in the period 2007–2013 (Fig. 3a), and the increased abundance of NEAC (Fig. 3b), the NDF decided that in the period 2014–2017, it was only necessary to sample the catch in the early part of the fishery period until the fraction of NEAC stabilized above 70%.

After 2011, when the “Henningsværboxen” area was opened for the first time during a brief period, it remained open most of the fishing season due to the large fraction of NEAC in the region. From 2015, the monitoring was based on previous years observations, and ended when the fraction of NEAC was above the 70% line in two consecutive samples.

## 4. Discussion

To our knowledge, this is the first example of a non-salmonid and commercially significant marine fishery being controlled by genetic methods “real-time”. Based upon the analysis of the *PanI* locus in Atlantic cod sampled from the commercial catch in Lofoten, which is the largest remaining cod fishery in the Atlantic, the proportion of NEAC was estimated within 24 h. This was conducted weekly in the late winter/early spring over an 11-year period, providing the NDF with the potential to quickly regulate the fishery where both NEAC and NCC overlap on the spawning grounds. In turn, this program has permitted commercial harvest of the highly abundant NEAC resource, while simultaneously limiting the impact on the more fragile NCC component. The total value of the landings in this fishery during the study period was approximately €730 million (The Norwegian Fishermen’s Sales Organization: [http://www.rafisklaget.no/portal/pls/portal/PORTAL.RPT\\_FANGST\\_AAR\\_SKREI.show\\_parms](http://www.rafisklaget.no/portal/pls/portal/PORTAL.RPT_FANGST_AAR_SKREI.show_parms)), while the overall cost for the presented genetic management regime (sampling, DNA extraction and analyses) was estimated to approximately 150 000 €. Thus, this study also demonstrates the economic viability of this type of management regime.

### 4.1. “Real-time” fisheries management

While there has been an almost exponential increase in the number of studies delineating population-genetic (and genomic) structure in marine organisms, there are still very few examples of commercial fisheries being routinely monitored or controlled by DNA-based methods “real-time”. This is despite the fact that the cost of genetic analyses continues to plummet, and that there is an ever-increasing availability of diagnostic or highly informative population-specific markers that can be used to permit identification of stocks and populations in potentially mixed fisheries (McKinney et al., 2017; Benestan et al., 2015; Larson et al., 2014). Thus, the genetic management program presented here provides a good illustration of the way in which other marine fisheries can potentially be managed “real-time” with

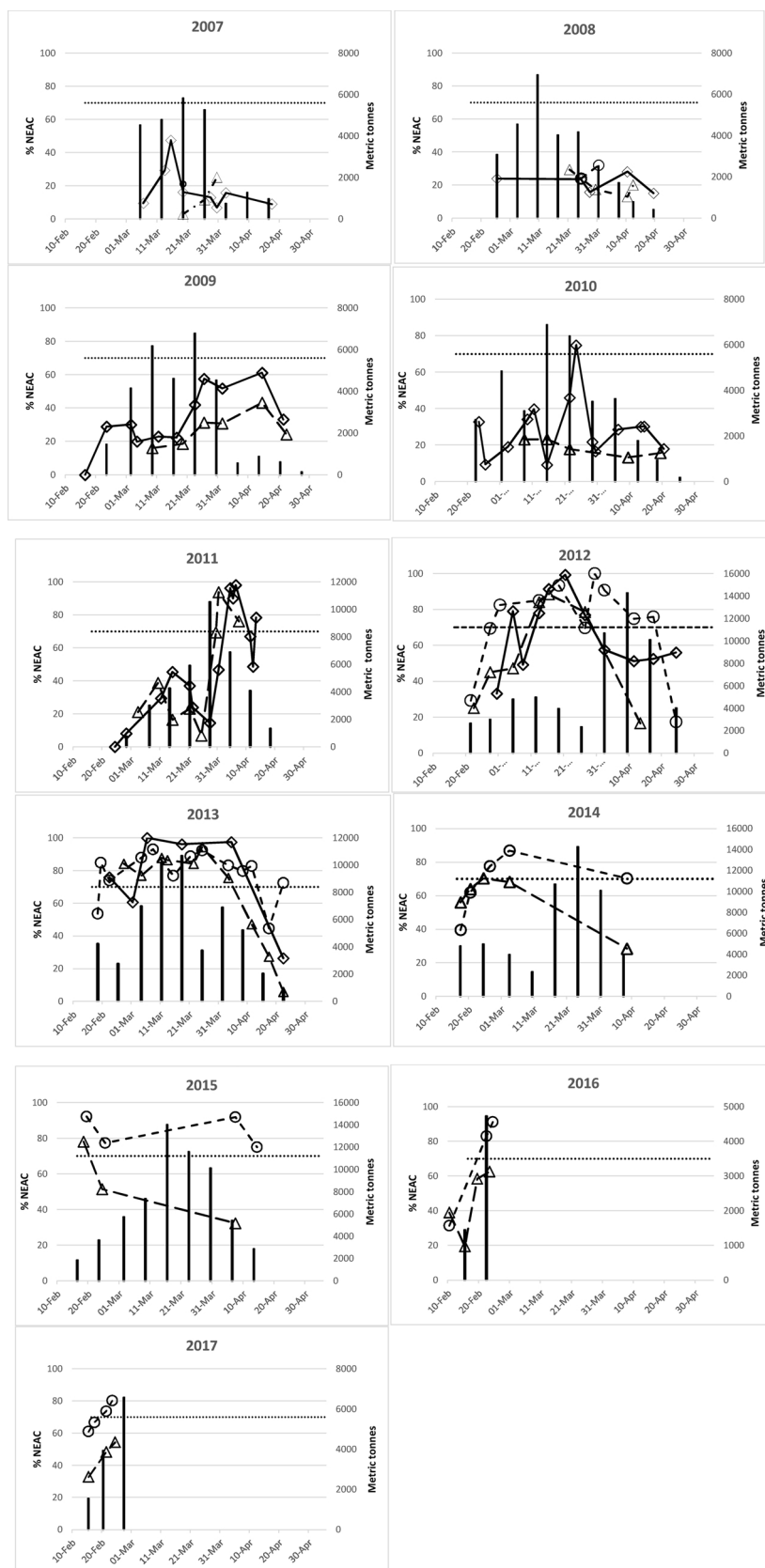
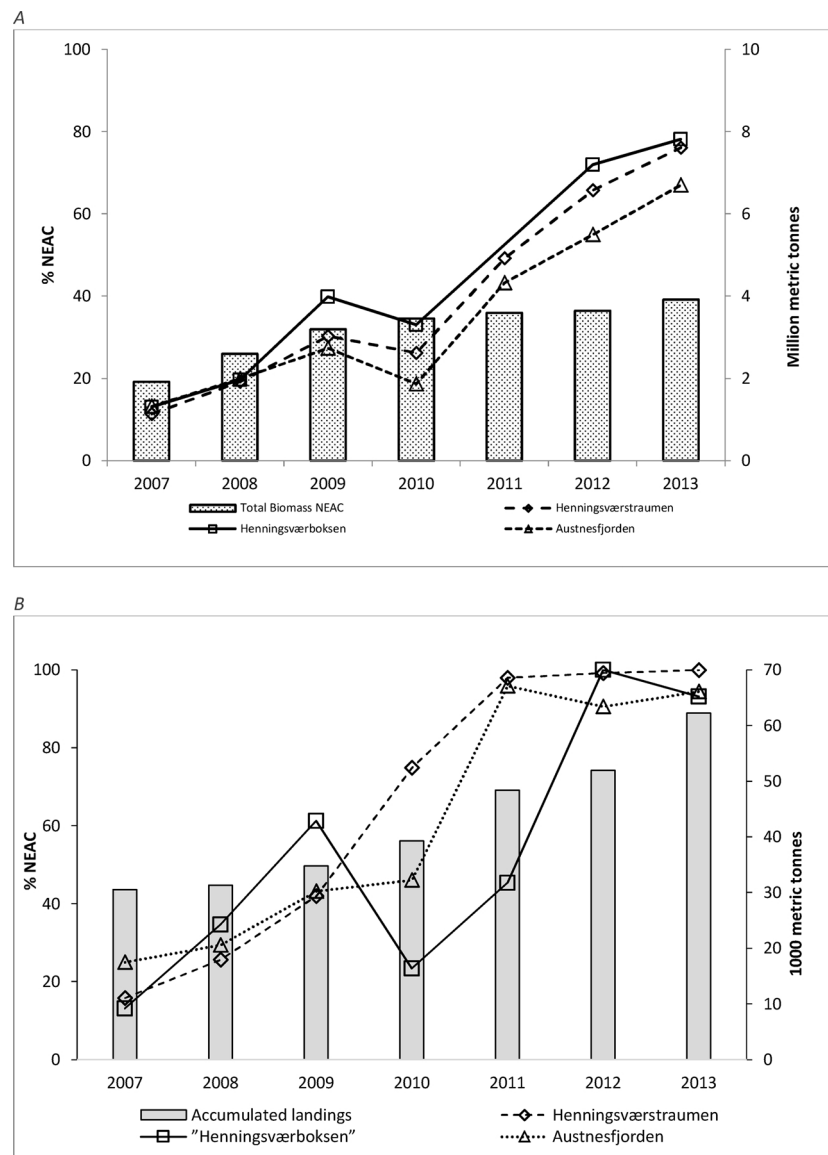


Fig. 2. Proportion NEAC in the commercial catches from the Lofoten islands in the period 2007–2017.  
 ◆: Henningsværstraumen, ▲: Austnesfjorden, ○: Henningsværboksen. The 70% line (-----) indicates the limit where the management will consider opening the closed area for commercial fisheries.



**Fig. 3.** A) Averaged fraction of NEAC in the landings in the three sampling areas during the sampling periods. The “total biomass NEAC” is the estimated biomass in million tons. B) Highest estimated frequency of NEAC in the sampled landings each year in the three sampling area. “Accumulated landings” are total cod landings in the Lofoten area in 1000 metric tons. Due to the stop in weekly monitoring from 2014, only data in the period 2007–2013 is presented.

genetics tools. Given that many of the world’s fisheries are or have been over-exploited, and that IUU fishing represents a massive challenge to sustainable fisheries management globally (FAO, 2016; Agnew et al., 2009), a similar approach to that described here has the potential to contribute to sustainable exploitation of other marine resources also.

One of the pre-requisites for using genetic methods to actively regulate a fishery is that informative or diagnostic markers, permitting identification of the separate components of the fishery, exist. I.e., markers that can distinguish between different species and cryptic species, populations and stocks, and wild vs. cultured and potentially domesticated fish released deliberately or inadvertently into the wild. In general, there is an increase in genetic structure among fish populations from marine to anadromous, and thereafter to freshwater species. Thus, it is perhaps not surprising that in addition to the high commercial and social value of salmonid fishes in general, there is a bias towards the best previously known examples of mixed fisheries being investigated with genetics methods for salmonids (that display an anadromous life-cycle and thus distinct population-genetic differentiation) (Bradbury et al., 2016; Gilbey et al., 2017; Ensing et al., 2013). Furthermore, it is the mixed-population salmonid fisheries in the Pacific

that have provided the best previously-documented examples of fisheries that have been monitored and regulated actively by genetics methods (Shaklee et al., 1999; Withler et al., 2004).

In addition to the examples of genetic analyses actively regulating salmonids fisheries in the Pacific, and NEAC and NCC in the Lofoten area described by the present work, there are examples other “fisheries” that have or are being actively managed by genetic methods in the Atlantic (but not necessarily “real-time”). These include identification of the farm of origin for domesticated escaped Atlantic salmon (Glover et al., 2008), Atlantic cod and rainbow trout (*Oncorhynchus mykiss*) captured in the sea (Glover, 2010; Glover et al., 2010; Glover et al., 2011), management of NCC and NEAC in the marine protected area developed in Borgundfjord, western-Norway (Johansen et al., 2017) and the minke whale (*Bonaerensis acutorostrata*) DNA register (Glover et al., 2012). The latter of which involves an individual database, upgraded by genotyping the entire catch yearly, that tracks whale meat at the individual sample level into the market-place in Norway, and when exported to Japan.

#### 4.2. Specifics of the Lofoten fishery management program

In order to permit the commercial exploitation of NEAC, while preventing over-exploitation of NCC, the NDF decided that the closed areas in Lofoten (“Henningsværboksen”) could be re-opened if the proportion of NEAC was above 70% for an extended period. This decision accentuated the need for reliable and “real-time” estimates of the proportion of the two stocks in the commercial catches. For this purpose, the genetic program implemented in the period 2007–2017 has played a decisive role.

The outer region of Lofoten is known to represent the main spawning ground for NEAC. In particular, Værøy, Røst and Bleiksegga (Fig. 1) are well-known spawning areas which also lay in the path of NEAC that may also migrate further south to spawn. In 2007 and 2008, the samples from these three locations contained between 94 and 100% NEAC in the landings in mid-March (Table 2). At the same time, the NEAC fraction in the samples obtained close to “Henningsværboksen” did not exceed 25%. The data also show large variation in the fraction of NEAC within each year during the season from February until late April, when the NEAC component appeared to leave the inner areas (Fig. 2). From the start of the monitoring in 2007, an increase in the fraction of NEAC caught in the Lofoten area, was observed over the years (Fig. 3a, b). This increase is also reflected in the total landings within the Norwegian zone of Atlantic cod north of 62°N, from approximately 210 000 t in 2007 to more than 460 000 t in 2013 (Anon., 2014), as well as an increase in total annual Norwegian quotas during this period (190 000–451 000 t). Thus, the increase in the fraction of NEAC observed in all three sampling areas in Lofoten (Fig. 3a); west of the closed area (Henningsværstraumen), east of the closed area (Austnesfjorden) and in the closed area itself (“Henningsværboksen”) appear to be the result of the increased abundance of NEAC in general. Clearly, the increased abundance of NEAC has meant that many of these fish now “spill-over” into the inner areas of Lofoten, whereas previously this area typically displayed only low to modest fractions of NEAC.

The observed fraction of NEAC in the Lofoten area increased from approximately 13% in 2007, to more than 90% for an extended period in 2013 in the spawning period (Fig. 3a). In 2014, the amount of NEAC migrating towards the Lofoten area from the Barents Sea was estimated to be high (IMR survey following the component into the spawning area). The projection from this survey was subsequently confirmed when the first samples analysed as part of the genetic monitoring program were analysed (Fig. 2). Furthermore, the fraction of NEAC in the inner-areas of Lofoten remained high throughout the spawning and fishing season (Fig. 2). Based upon the observed large fractions of NEAC in the period 2011–2014, the closed area on the inside of Lofoten (“Henningsværboksen”), was opened for commercial fishing for approximately one week in 2011, two weeks in 2012, and for the whole season in 2013 and 2014. Periods with both high and low fractions of NEAC in this region have previously been observed, and although the exact ecological reasons for these changes in spawning migrations for NEAC are not clear (Höffle et al., 2014), they appear linked with the increase in NEAC in general (Fig. 3a, b).

Recent declines of NCC has put focus on the potential negative effects of previous management regimes implemented in this area. Previously, “Henningsværboksen” was closed for all commercial vessels except hand line and fishing rod was put in operation before the 2005 spawning season to reduce the fishing on the vulnerable NCC stock. This is an area where concentration of spawning NCC is observed and the fishing activity historically has been high. It is a popular area for the local fishing fleet as the distance from land is short. Since NCC are harvested under a merged NCC/NEAC quota, the annual regulations of all cod fisheries along the coast is now aimed at moving part of the traditional coastal fishery (vessels using Danish seine and vessels larger than 15 m) from catching coastal cod in the fjords to a cod fishery outside the fjords, where the proportion of NEAC is higher during the spawning season. Further restrictions were also introduced in 2007, not

allowing pelagic gillnet fishing for cod and reducing the allowed by-catch of cod when fishing for other species inside fjord lines from 25% to 5%, and outside the fjord lines from 25% to 20%.

#### 4.3. Use of *PanI* to estimate the fraction of NEAC

The “real-time” genetic monitoring program detailed here used the *PanI* marker to estimate the proportion of NEAC in the mixed cod fishery. *PanI* has previously been demonstrated to provide close to diagnostic separation between NEAC and NCC (Fevolden and Pogson, 1997; Westgaard and Fevolden, 2007), and given its ease of analysis, has permitted rapid estimation of NEAC in the commercial catch within 24 h of landing.

Ever since Rollefson (1933) reported differences in the otolith structure between NCC and NEAC, there have been discussions whether the NEAC and NCC reflect different species, populations, or components of the same population/stock. Early studies employing allozyme and mtDNA markers revealed limited genetic variation between NEAC and NCC (Jørstad, 1984; Mork et al., 1985; Smith et al., 1989; Dahle, 1991; Arnason et al., 1992). In contrast, analyses based upon microsatellites, Haemoglobin (*HbI*) and Pantophysin (*PanI*) have revealed highly significant genetic differentiation between NEAC and NCC (Dahle and Joerstad, 1993; Fevolden and Pogson, 1997; Hutchinson et al., 2001; Skarstein et al., 2007). More recent studies, based upon single nucleotide polymorphisms (SNPs) (Hemmer Hansen et al., 2013; Karlsen et al., 2013), revealed that genetic differentiation between the long-migratory and short-migratory “ecotypes” (i.e., NEAC and NCC respectively) is primarily located in specific areas of the genome. These suggestions have subsequently been confirmed, with large chromosomal inversions being observed between NEAC and NCC on several linkage groups (i.e., chromosomes) (Berg et al., 2016; Kirubakaran et al., 2016). Kirubakaran et al. (2016) further suggested that the adjacent inversions on Linkage group 1 may act as a supergene in the NEAC, where recombination is effectively blocked. It is within one of these two adjacent inversions located on linkage group 1 that the *PanI* gene lies. Thus, although the *PanI* gene itself may or may not be part of the target of selection within the inversion, it is nevertheless hitch-hiking with a strong selection force. Given that inversions effectively block recombination within-type, this has contributed to the highly divergent allele-frequency differences between NEAC and NCC utilised here.

Even though the *PanI* gene is located in a genomic area that is under selection, several studies have shown that the allele frequency differences between NCC and NEAC are temporally stable. Fevolden et al. (Fevolden and Pogson, 1997; Sarvas and Fevolden, 2005) found a significantly higher frequency of the *PanI*<sup>A</sup> allele in the NCC samples (on average 0.81) compared to NEAC (on average 0.10) collected from the Barents Sea. Analyses of age 1+ cod collected in the Lofoten area, Vesterålen and Røst (Fig. 1) in 1997, 1999 and 2000 indicated temporal stability of the *PanI* gene and did not indicate any extended mixing between individuals with a high *PanI*<sup>A</sup> frequency (NCC) and individuals with a high *PanI*<sup>B</sup> frequency (NEAC). Furthermore, Wennevik et al. (2008) performed a comprehensive study of cod from around the Lofoten Islands using three types of genetic markers (*Pan I*, microsatellites and *HbI*), revealing similar significant differences between the NEAC and NCC (Wennevik et al., 2008). While the use of genetic markers potentially under selection to investigate population connectivity was traditionally viewed as sub-optimal, and thus discouraged, contemporary opinion is that markers under selection may indeed provide very significant advantages over neutral markers for identification and monitoring marine fish populations on a contemporary time-scale (Ferguson, 1994). Indeed, target identification of “outlier” markers to enable identification of populations and stocks is actively being pursued by many (e.g. Freamo et al., 2011; Karlsson et al., 2011; Milano et al., 2014).

Within both NEAC and NCC, *PanI*<sup>AB</sup> heterozygote individuals are observed in very low frequencies. Here, in order to remove the





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